Diet composition affects the rate and N:P ratio of fish excretion

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SUMMARY

1. Nutrient recycling by fish can be an important part of nutrient cycles in both freshwater and marine ecosystems. As a result, understanding the mechanisms that influence excretion elemental ratios of fish is of great importance to a complete understanding of aquatic nutrient cycles. As fish consume a wide range of diets that differ in elemental composition, stoichiometric theory can inform predictions about dietary effects on excretion ratios.
2. We conducted a meta-analysis to test the effects of diet elemental composition on consumption and nutrient excretion by fish. We examined the relationship between consumption rate and diet N:P across all laboratory studies and calculated effect sizes for each excretion metric to test for significant effects.
3. Consumption rate of N, but not P, was significantly negatively affected by diet N:P. Effect sizes of diet elemental composition on consumption-specific excretion N, P and N:P in laboratory studies were all significantly different from 0, but effect size for raw excretion N:P was not significantly different from zero in laboratory or field surveys.
4. Our results highlight the importance of having a mechanistic understanding of the drivers of consumer excretion rates and ratios. We suggest that more research is needed on how consumption and assimilation efficiency vary with N:P and in natural ecosystems in order to further understand mechanistic processes in consumer-driven nutrient recycling.

Introduction

Consumers can play an essential role in nutrient cycles in marine and freshwater ecosystems by controlling the storage and fluxes of key nutrients such as nitrogen (N) and phosphorus (P) (Kitchell *et al.*, 1979; Elser *et al.*, 1988; Vanni, 2002; McIntyre *et al*., 2007; Allgeier, Yeager & Layman, 2013). Through the excretion of dissolved inorganic nutrients, consumers can supply significant amounts of limiting nutrients to primary producers and decomposers (McIntyre *et al*., 2008; Small *et al*., 2011). While a considerable body of literature has developed around investigations of the importance of consumers to nutrient cycles in aquatic ecosystems, a mechanistic understanding of what influences rates and elemental ratios of nutrients excreted by consumers has lagged behind. Consumers can create biogeochemical hotspots simply by achieving locally high biomass (McIntyre *et al.*, 2008; Atkinson *et al.*, 2013; Capps & Flecker, 2013a), but the digestion, metabolism, storage and retention of consumed nutrients in consumer bodies, in combination with overall biomass, control the role individual species play in altering ecosystem function (Vanni *et al.*, 2002; Small *et al.*, 2011; Capps & Flecker, 2013b; Vanni, Boros & McIntyre, 2013). As a result, both the elemental composition of an organism and its diet should impact the rates and ratio at which it excretes nutrients (Sterner, 1990; Elser & Urabe, 1999; Sterner & Elser, 2002). While the effect of organismal elemental composition on nutrient recycling by aquatic vertebrates has been investigated (e.g., Vanni *et al.*, 2002; Hood, Vanni & Flecker, 2005), empirical studies of the impacts of diet elemental composition on excretion ratios have provided mixed results. The positive relationship between diet N:P and excreted N:P predicted by Sterner (1990) has been found in *Daphnia*, crayfish and mottled sculpin (*Cottus bairdi*) (He & Wang, 2008; McManamay *et al.*, 2011), but no significant relationship has been found for a number of other species of fish and invertebrates (Schindler &

Eby, 1997; Verant *et al.*, 2007; McManamay *et al.*, 2011; Taylor *et al.*, 2012). We investigate the impacts of diet on consumer excretion ratios in fish, a group of consumers that is both abundant in aquatic ecosystems and exhibits a great diversity of dietary strategies over which to examine excretion responses.

Fish are both abundant and diverse in many aquatic ecosystems, and as a result they have been frequently identified as the most important nutrient recyclers or retainers in a diverse range of aquatic systems (e.g., McIntyre *et al.*, 2007; Small *et al.*, 2011; Allgeier *et al.*, 2013; Capps & Flecker, 2013b). Fish are diverse taxonomically as well as functionally, with known diets ranging widely in elemental composition from plant and algal detritus to invertebrates and other vertebrates (González-Bergonzoni *et al.*, 2012). While some fish species are highly specialized to feed on specific foods, many fish are omnivorous to some degree and thus may consume diets that vary widely in quality over time or ontogeny (e.g., Grimm, 1988; Pilati & Vanni, 2007; González-Bergonzoni *et al.*, 2012). Diets that are animal-based are generally relatively higher in P content than plant- or algae-based diets (e.g., Green, Hardy & Brannon, 2002), thus the impacts of animal- vs. plant- or algae-based diets on organismal physiology are informed by the mass balance of multiple chemical elements and energy in ecological systems employed by ecological stoichiometry (Sterner & Elser, 2002). Following a mass balance model of fish growth assuming no difference in growth rate between diets, the difference between the amount of a given nutrient in the diet and that used by the consumer will equal the total released, which includes both nutrients excreted as dissolved inorganic and organic molecules and those egested as particulate waste (Kitchell *et al.*, 1974; Sterner, 1990; Schindler & Eby, 1997; Fig. 1). Therefore, fish excretion ratios should be proportional to diet elemental composition across a gradient of food elemental ratios unless fish differentially assimilate N and P (Sterner, 1990;

Schindler & Eby, 1997; Sterner & Elser, 2002). However, if fish differentially excrete and egest waste products, these ratios may not be directly proportional. Such a scenario arises when assimilation efficiency changes with diets of varying composition.

To assess how diet composition affects fish excretion ratios, direct manipulations of organismal diets in a controlled setting are required. Here we review the literature for studies in which multiple diets were fed to fish in a controlled setting and consumption rates and excretion rates and/or ratios were measured. Specifically, we draw on the field of experimental aquaculture research which represents a rich source of data on physiological responses of consumers to differing diets, the value of which is only beginning to be recognized by ecologists (Boersma & Elser, 2006; Benstead *et al.*, in press). We employ a meta-analysis using standardized effect sizes to quantify how both consumption and composition of diet may affect excretion ratio in fish. Finally, we discuss the implications of the results from controlled settings to nutrient recycling in natural ecosystems.

Methods

We used a meta-analytic approach to determine if fish consumption rates and nutrient excretion ratios are influenced by the N and P composition of their diet. We used the ISI Web of Science database to search the peer-reviewed literature for studies of fish where diet was directly manipulated and a dissolved excretion response was measured. While faecal egestion is undoubtedly important in the N and P budgets of organisms (Fig. 1; Halvorson *et al.*, 2015), we focus on dissolved excretion because it is in this form that excreted nutrients can have significant immediate impacts at the ecosystem scale (e.g., Kitchell *et al.*, 1979; McIntyre *et al.*, 2008; Small *et al.*, 2011). We included studies that measured mass-specific excretion as a rate and

those that reported it only as a loading per unit of fish biomass. We performed this search using the terms *fish, diet* and *excretion*. Our search included articles published between 1970 and 2013. This search initially returned >600 articles, which were cursorily examined by title to determine whether they were likely relevant to the meta-analysis; for example, articles discussing only modeled excretion and growth or the use of fishmeal as a feed for other animals were disregarded. We identified 74 articles that appeared to be relevant by suggesting some type of study of fish N and P excretion among different diets which were then searched in greater detail to determine whether they met our criteria of inclusion. Studies included in the meta-analysis were those that were conducted on fish from a single population, included multiple diets that were directly manipulated or measured over natural gradients, measured N and P composition and fish consumption rates of those diets and measured N and/or P excretion in some form. In the few instances where our search returned multiple studies of a single species by the same research group, we selected only one of them with a random number generator to avoid violating test assumptions of independence. We categorized studies as those with direct diet manipulations in laboratory settings and those that measured natural variation diets in field settings and also noted whether dietary P was manipulated by varying the level of organic or inorganic P. We found no studies that conducted diet manipulation experiments in a natural setting.

As raw excretion rates may be influenced by differences in diet elemental composition and changes in consumption rate caused by diet differences, we used linear models of mass-specific consumption rate ($g * g\ fish^{-1} * day^{-1}$) of N, P and total food consumption predicted by diet N:P to calculate and test for significance of effect sizes. From these models, we calculated effect size as the Pearson correlation coefficient *r*, which we transformed to *Z*-scores using the Fisher transformation (Rosenthal & DiMatteo, 2001). Then, we tested whether mean effect sizes

differed from 0 using *t*-tests with Bonferroni corrections to adjust α when performing multiple comparisons with the same dependent variable (Rice, 1989; Rosenthal & DiMatteo, 2001). We then calculated consumption-specific excretion measurements for each study by dividing N, P and N:P excreted by the mass-specific consumption rate when feeding on a given diet and used the above methods to calculate effect sizes for both consumption-specific and raw N, P and N:P excreted as a response to diet N:P in diet manipulation studies. Field surveys did not measure consumption rates and some did not report N and P excretion data individually, thus we could not calculate consumption-specific and single nutrient excretion effect sizes for those studies.

To assess whether effect sizes may have been influenced by other factors aside from diet composition, we tested study heterogeneity in the effect size measures. First, we used Cochran's *Q* to test for significance of study heterogeneity for each effect size measure. Cochran's *Q* follows a χ^2 distribution and is a widely used and relatively conservative test of study heterogeneity in meta-analyses (Takkouche, Cadarso-Suarez & Spiegelman, 1999). For those effect sizes with significant heterogeneity, we fit linear regression models for each effect size measurement as a response to difference in N:P between the diet end-members, average water temperature during the experimental period, initial fish mass and experimental duration (Rosenthal & DiMatteo, 2001). Our sample size was not sufficient to estimate the interaction terms between all of these variables thus we examined only main effects. We assessed homoscedasticity and normality of residuals visually for each model with a plot of model residuals vs. fitted values and a normal probability plot, respectively. We could not construct linear regression models for field studies due to a lack of data presented in those manuscripts and small sample size. All analyses were conducted in the software R v2.15 (R Core Team, 2013).

Results

Of the 74 candidate papers identified as possibly relevant, we found 19 independent studies that met our criteria for inclusion in the meta-analysis (Table 1). Of these, two studies featured only two experimental diets; these studies were excluded from the meta-analysis because effect sizes could not be calculated from two data points. Of the remaining 17 studies, 15 were diet manipulation experiments conducted in controlled laboratory facilities and two were field surveys conducted over natural gradients of diet elemental composition. Of the diet manipulations, 12 studies manipulated the levels of animal vs. plant-based protein while three studies directly manipulated dietary P content by adding phosphate salts to the same base diet; however these three studies did not measure N excretion. The majority of laboratory studies fed fish to apparent satiation, although several fed fish at specific levels based on fish body mass (Ballestrazzi *et al.*, 1994; Green *et al.*, 2002; Sumagaysay-Chavoso, 2003; Yang *et al.*, 2011). The laboratory studies included involved 10 fish species in seven families while the field studies included involved seven fish species in seven families (Table 1). Resource N:P ratios (by mass) ranged from 2.5 to 56 in laboratory studies (mean=8.2, SD=8.3) and from 2.4 to 174 in field studies (mean=44.7, SD=42.4). All field studies measured excretion N:P, but only 12 of 15 laboratory studies presented N excretion data that allowed us to calculate N:P ratios of excretion. Additionally, all laboratory studies measured average initial fish mass, the average water temperature and the length of the experimental period between when the diet switch began and when excretion was measured.

We first examined whether consumption rates differed with diet composition. Total mass-specific consumption was not significantly affected by diet N:P (two-tailed *t*-test, t=-1.796, v=11, P=0.10). Mass-specific consumption rate of N was also unaffected by diet N:P (two-tailed *t*-test,

t=-0.270, ν=11, P=0.480) across studies but mass-specific P consumption rate significantly decreased with increasing diet N:P (two-tailed t-test, t=-3.650, ν=11, P=0.004) (Fig. 2).

Diet effects on excretion ratios were similar for laboratory and field studies; however we had fewer results for field studies due to the lack of consumption and separated N and P excretion data. For diet manipulation studies, effect size of diet N:P was significantly below 0 for P excretion (two-tailed t-test, t=-2.606, ν=14, P=0.021), and positive, but not significantly different from 0 for N excretion (two-tailed t-test, t=1.381, ν=11, P=0.195) (Fig. 3). However, effect sizes for consumption-specific excretion of both P (two-tailed t-test, t=-2.244, ν=14, P=0.042) and N (two-tailed t-test, t=2.915, ν=11, P=0.014) were significantly different from 0 (Fig. 3). Mean effect size of diet elemental composition on excretion N:P was not significantly different from 0 in diet manipulation studies (two-tailed t-test, t=2.00, ν=11, P=0.071) nor field surveys (two-tailed t-test, t=-0.002, ν=6, P=0.999), but was significantly different from 0 when corrected for consumption in diet manipulations (two-tailed t-test, t=2.42, ν=11, P=0.034) (Fig. 4). Of all excretion response effect sizes in diet manipulation studies, only raw P excretion exhibited significant heterogeneity (Q=23.82, ν=11, P=0.014). However, this heterogeneity was not significantly related to temperature, body mass, experimental duration or the difference in diet elemental composition (P>0.35 for all slopes). Additionally, there was significant heterogeneity in the response of N:P excretion in field studies (Q=12.83, ν=6, P=0.046), but we could not further explore any potential sources of this heterogeneity with the data available.

Discussion

In this study we synthesize a variety of empirical studies to show that diet can influence the ratio of dissolved nutrients excreted by aquatic consumers and suggest mechanisms by which

192 it may do so. We found that dietary composition can have significant impacts on fish excretion
193 ratios in controlled aquaculture settings. In particular, fish feeding on low N:P diets with higher
194 amounts of animal protein excreted at a lower N:P ratio when accounting for the amount
195 consumed (Fig. 4). While these effects were strong in laboratory studies, other sources of
196 variation must be examined to improve our mechanistic understanding of consumer-driven
197 nutrient recycling in the field.

198 The mass-balance used in ecological stoichiometry (Sterner & Elser, 2002) provides a
199 simple framework for making predictions about organismal growth and nutrient recycling (Elser
200 *et al.*, 1988; Sterner, 1990; Elser & Urabe, 1999; Elser, Hayakawa & Urabe, 2001). In a mass-
201 balance model of organismal growth, an animal should excrete and/or egest the excess nutrients
202 consumed beyond what is needed for somatic growth and reproduction (Kitchell *et al.*, 1974;
203 Sterner & Elser 2002; Fig. 1). As animals often exhibit strong stoichiometric homeostasis, their
204 body elemental composition should not change substantially with diet; therefore excess
205 consumed nutrients should be excreted or egested (Sterner & Elser, 2002). Some recent studies
206 have suggested fish can be stoichiometrically flexible in some cases (McManamay *et al.*, 2011;
207 El-Sabaawi *et al.*, 2012a,b; Benstead *et al.*, in press), thus offering a potential explanation for the
208 lack of strong correspondence of diet to excretion ratios in prior field studies (Schindler & Eby,
209 1997; McManamay *et al.*, 2011). However, in finding that consumption-specific excretion of N
210 and N:P increases and P decreases with increasing diet N:P, our results support the predictions of
211 stoichiometric theory. By accounting for consumption rates, we have gained new insights into
212 how diet affects excretion ratios, insights that we could not from field studies for which
213 consumption is extremely challenging to measure.

Our results highlight the importance of consumption to excretion ratios. Most importantly, we found that while fish excretion rate of N did not significantly differ with diet composition, the excretion rate of N per gram of food consumed did (Fig. 3). In contrast, excretion of P significantly decreased with increasing dietary N:P both independent of consumption and per gram consumed (Fig. 3). This result could stem from fish eating less total food when feeding on high N:P diets and/or the fact that those diets had less P. The fact that mass-specific P consumption declined with increasing diet N:P is likely a consequence of most studies manipulating diet N:P primarily by manipulating P rather than N contents. As dietary P contents of fish can vary substantially through space and time (e.g., Mehner *et al.*, 1998; Zandonà *et al*., 2011), this mechanism certainly impacts fish excretion ratios in natural settings. Further, mass-specific consumption rates tended to decline with increasing dietary N:P (P=0.10), thus this mechanism may be important in some, but not all situations. If fish consume less material when feeding on high N:P foods, and they also excrete more N and less P per gram of diet consumed, then the ratio of N:P excreted will be altered through both direct and consumptive effects of diet stoichiometry. However, the underlying fact that both N and P excretion per gram consumed differed with diet N:P ratio is itself an interesting result that merits further examination.

In many of these studies, and often in natural systems, shifts in diet elemental composition co-occur with differences in the abundance of animal, plants or algae in the diet. In systems where consumers are largely consuming entirely one group of diet items, such as zooplankton feeding on phytoplankton, dietary N:P alone should largely determine how diet impacts excretion ratios (e.g., Sterner, 1990). However, when animals consume diets with co-varying elemental composition and protein sources, these confounding sources of variation can

237 produce differing effects on excretion ratios. Differences in the biochemical form of nutrients
238 present could alter assimilation efficiency, which could in turn lead to differential egestion and
239 excretion of individual nutrients. Although previous researchers have assumed constant
240 assimilation efficiencies across diets in fish, this assumption is unrealistic for fish that consume
241 diets consisting of multiple food types (Lall, 1991). Since excess undigested nutrients should be
242 egested as particulate waste products (Wotton & Malmqvist, 2001; Halvorson *et al.*, 2015),
243 concurrent changes in digestibility with diet N:P could confound effects of diet on dissolved
244 excretion rates. For example, variation in protein digestibility among plant- or algae-based and
245 animal-based diet items could lead to differences in the amount of N egested as opposed to
246 excreted without substantially affecting the amount of P egested or excreted (Robbins *et al.*,
247 2005). However, P digestibility often differs between plants, algae and animals because plants
248 often contain large amounts of P in phytate or phytic acid, which is difficult for many fish to
249 digest (Lall, 1991). In our study a large number of plant-based diets were treated with phytase to
250 increase P digestibility, thus we expected effects of P digestibility to be lower in magnitude than
251 those of N digestibility. However, this digestibility difference is likely important to consumers in
252 natural settings where fish cannot easily digest phytic acid. Our results support this prediction, as
253 consumption-specific excretion rates of both N and P differed with diet N:P (Fig. 3), suggesting
254 that N and P assimilation efficiency differed when feeding on high N:P plant-based diets vs. low
255 N:P fishmeal-based diets. If the proportion and elemental ratios of material egested and excreted
256 differ as a function of diet elemental composition and/or protein source, no strong relationship
257 between diet elemental composition and excretion ratios may be observed (McManamay *et al.*,
258 2011). As a result, our results support the idea that factors other than diet N:P such as protein

digestibility, phytate contents and consumption rates must be taken into account when assessing the impacts of diet on consumer excretion ratios.

In spite of the considerable interest in excretion ratios such as N:P due to the importance of stoichiometric ratios of nutrients supplied to primary producers (e.g., Elser *et al.*, 1988; Sterner, Elser & Hessen, 1992), studies of excretion ratios are complicated by the fact that physiological regulation of N and P is largely controlled separately in fish. The majority of P consumed by fish and other vertebrates is used for bone mineralization (Lall, 1991; Hendrixson *et al.*, 2007; Huitema *et al.*, 2012), yet a large amount of N consumed is used for the synthesis of protein (Sterner & Elser, 2002). However, stoichiometric theory offers a link between these disparate physiological pathways. Since fish are generally stoichiometrically homeostatic over an individual life stage (Sterner & Elser, 2002), those excess nutrients not assimilated must be excreted and/or egested. Therefore, the ratio of what is consumed to what is needed by a fish can still be used to predict excretion ratios even if the individual pathways of those elements within the organism are not tightly connected. Another potential factor that may confound dietary effects on excretion is that excretion rates of N and P scale differently with body mass (Torres & Vanni, 2007). If consumers grow at different rates when feeding on diets of differing elemental composition, differences in body mass alone could account for differences in excretion ratios (Villéger *et al.*, 2012a,b). We were unable to correct for the different allometries of N and P excretion because the units in which excretion was reported varied between studies, but all studies reported excretion as some function of fish mass. We believe that our conclusions are robust to the lack of an allometric correction in our analyses since specific growth rate was not significantly affected by diet N:P in the studies analyzed. However, P-limitation of growth in fish is possible at ecologically relevant dietary P levels (Hood *et al.*, 2005; Benstead *et al.*, in press),

thus we do believe that organismal growth and size differences caused by feeding on different diets could lead to differences in excretion ratios in natural settings.

Physiological responses to differing diets that are not accounted for in field studies of diet effects on excretion ratios may explain the difficulty of translating laboratory results into field settings. While heterogeneity in the only effect size measured in field studies, excretion N:P, was significantly greater than 0, only one of the six effect size measurements, raw P excretion, exhibited significant heterogeneity in laboratory studies. One source of this discrepancy may be the lack of correspondence between measured resources and actual fish diets. There are considerable difficulties associated with measuring the true elemental composition of the diet consumed and assimilated in the field. If the resources sampled by the researchers do not specifically match what the fish are consuming and assimilating, conclusions about the effect of diet on excretion ratios may be invalid (Hood *et al*., 2005). This may be particularly true of omnivorous fish, which may consume different proportions of animals, plants and algae at different sites or times of the year (e.g., Grimm, 1988). Further, local selection pressures such as the degree of predation can lead to differences in fish dietary habits and life history traits between sites (Zandonà *et al*., 2011; El-Sabaawi *et al.*, 2012a). While differences between fish in each treatment were controlled for in aquaculture studies by selecting all fish from one population, such as a single hatchery source and keeping all fish under the same conditions aside from the diet they were fed, field studies often compare individuals from separate populations.

Evolutionary differences between populations in the field studies may also represent a covariate that cannot be separated from diet differences, thus complicating interpretation. That is, comparisons of diet differences of a given species between sites, e.g., different streams or lakes, represent populations of that species that likely experience at least some degree of genetic

separation. Therefore, differences in genotypes between populations cannot be ruled out as a confounding variable in these studies. While stoichiometric theory predicts that individuals of a given animal species and life history stage should have a given C:N:P stoichiometric composition (Sterner & Elser, 2002), this does not apply across organisms with differing genotypes. Indeed, P homeostasis is known to be genetically controlled in developing fish (Huitema *et al.*, 2012). Therefore, differential selection pressures between populations may affect a fish's response to diet quality. Differences in selection pressures such as temperature, salinity, resource quality and predation pressure also drive evolution of organismal traits and life histories that can affect body elemental composition (e.g., Zandonà *et al*., 2011; El-Sabaawi *et al*., 2012a,b; Liess *et al.*, 2013). Since interpopulation differences may be a source of unmeasured variance in studies across natural gradients, linking evolutionary divergence to consumer-driven nutrient recycling represents a promising area of future research.

Since Vanni (2002) reviewed the importance of nutrient recycling by consumers in freshwater ecosystems, we have gained a greater appreciation for the role animals play in the way nutrients cycle through ecosystems. Indeed, many studies have investigated how important the transportation and transformation of nutrients by consumers can be to ecosystem function (McIntyre *et al.*, 2007; Layman *et al.*, 2011; Small *et al.*, 2011; Atkinson *et al.*, 2013). However, more work is needed to improve our understanding of the mechanisms that influence consumer excretion rates and ratios. Our results suggest that diet is one of these mechanisms, but relatively few studies have examined the effects of diet composition on consumer-driven nutrient recycling in the field (McManamay *et al.*, 2011). We show that dietary N:P can affect excretion ratios across several fish species when correcting for consumption (Fig. 4). As raw N excretion was not significantly affected by dietary N:P (Fig. 3), we hypothesize that differences in protein

digestibility can weaken the relationship between dietary N:P and excreted N:P for consumers that feed on both animal and plant or algal material. While the application of stoichiometric theory provides a promising framework through which to investigate consumer impacts on ecosystem function, effective testing of stoichiometric theory may require that future work examining dietary effects on excretion rates and ratios should consider not only dietary N:P but specifically the forms in which these nutrients are present in the diet, how much is consumed and how efficiently consumers assimilate dietary elements. Additionally, it is worth investigating whether evolutionary differences between populations impact intraspecific consumer nutrient recycling rates. While our study suggests that dietary composition can play a significant role in altering excretion rates and ratios, more careful tests of this effect in the field across a range of diets are needed before the impact of resource quality changes on consumer-driven nutrient recycling and its importance to ecosystem function can be fully understood and integrated into conceptual and theoretical frameworks.

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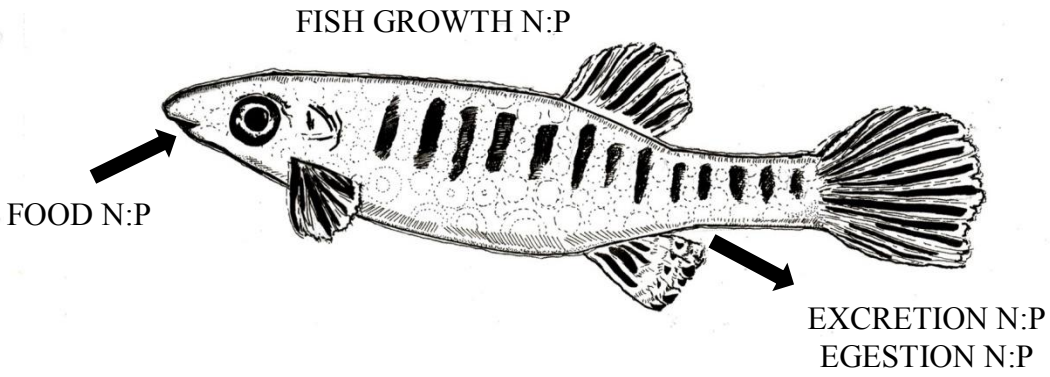
Table 1. Species and family identities of fish in studies included in the meta-analysis. Reference numbers are as follows: (1) Sukumaran *et al.*, 2009; (2) Sumagaysay-Chavoso, 2003; (3) Jahan *et al.*, 2002; (4) Kaushik *et al.*, 2004; (5) Ballestrazzi *et al.*, 1994; (6) Tantikitti, Sangpong & Chiavareesajja, 2005; (7) Yang *et al.*, 2009; (8) Green, Hardy & Brannon, 2002; (9) Bureau & Cho, 1999; (10) Rodehutscord, Gregus, & Pfeffer, 2000; (11) Hossain *et al.*, 2007; (12) Sarker, Satoh & Kiron, 2007; (13) Storebakken, Shearer & Roem, 1998; (14) Sarker *et al.*, 2011; (15) Dias *et al.*, 2009; (16) Small *et al.*, 2011; (17) McManamay *et al.*, 2011.

Species	**Family**	**Reference(s)**
Diet Manipulations		
Catla catla	Cyprinidae	1
Chanos chanos	Chanidae	2
Cyprinus carpio	Cyprinidae	3
Dicentrarchus labrax	Moronidae	4,5
Lates calcarifer	Latidae	6
Oncorhynchus mykiss	Salmonidae	7,8,9,10
Pagrus major	Sparidae	11,12
Salmo salar	Salmonidae	13
Seriola quinqueradiata	Carangidae	14
Sparus aurata	Sparidae	15
Field Studies		
Alfaro cultratus	Poeciliidae	16
Astatheros alfari	Cichlidae	16
Astyanax aeneus	Characidae	16
Atherinella hubbsi	Atherinopsidae	16
Chrosomus erythrogaster	Cyprinidae	17
Cottus bairdi	Cottidae	17
Oncorhynchus mykiss	Salmonidae	17

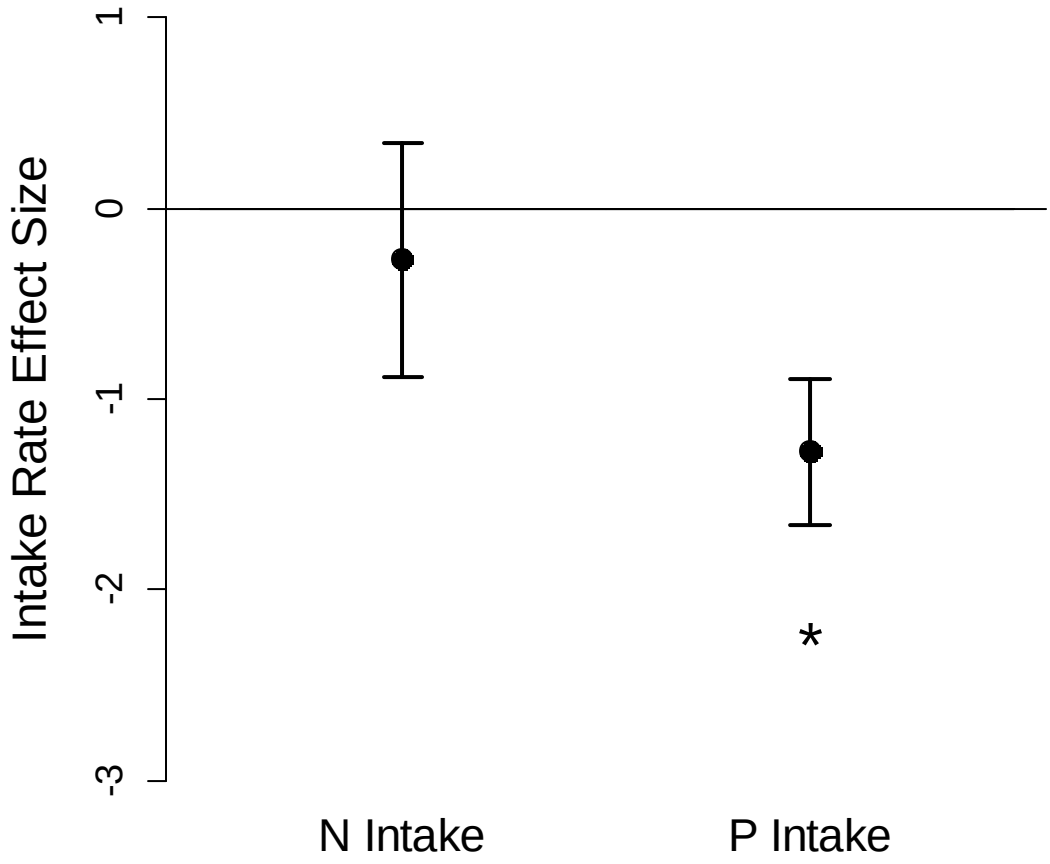


EXCRETION N = FOOD N – (FISH GROWTH N + EGESTION N)

EXCRETION P = FOOD P – (FISH GROWTH P + EGESTION P)

586
587 Fig. 1 Mass balance model of N and P budgets for a fish. Our model represents a conceptual
588 simplification of the major nutrient fluxes in consumers (Kitchell *et al.*, 1974; Sterner, 1990).

589



590

591 Fig. 2 Effect size of diet N:P on intake (g * g fish^{-1} * day^{-1}) of N and P in diet manipulation
592 studies. Effect size, η^2, was measured as the treatment sum-of-squares divided by total sum-of-
593 squares from a linear model then transformed into a *Z* score for ease of analysis. Bars with *
594 indicates effect size significantly different from zero based on a two-tailed *t*-test. Column lengths
595 indicate mean effect sizes and error bars represent 95% confidence intervals.

596

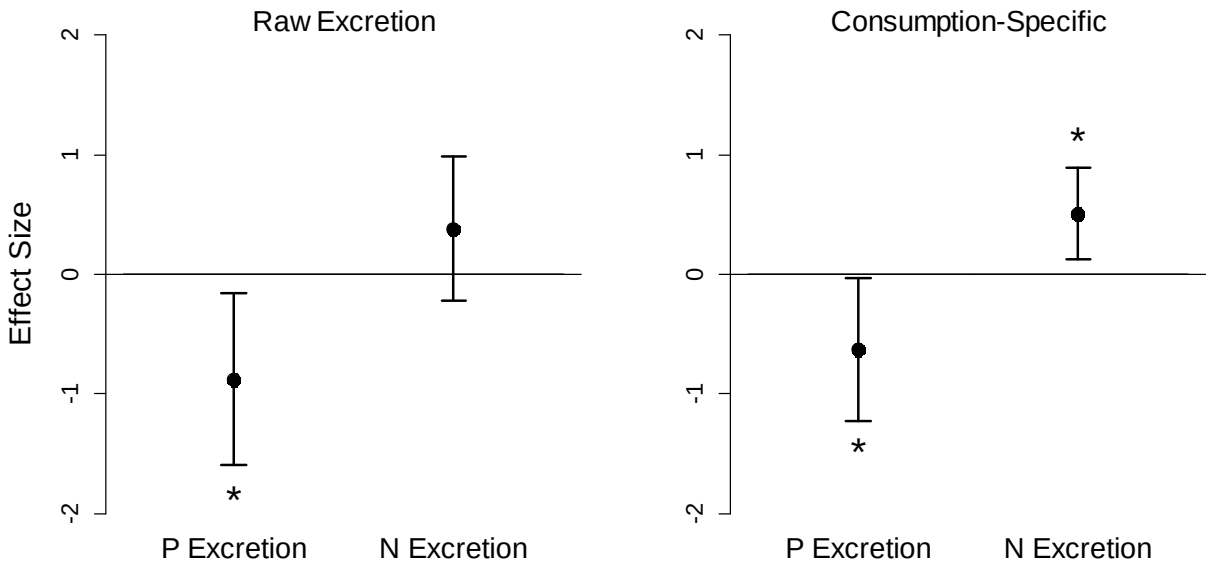
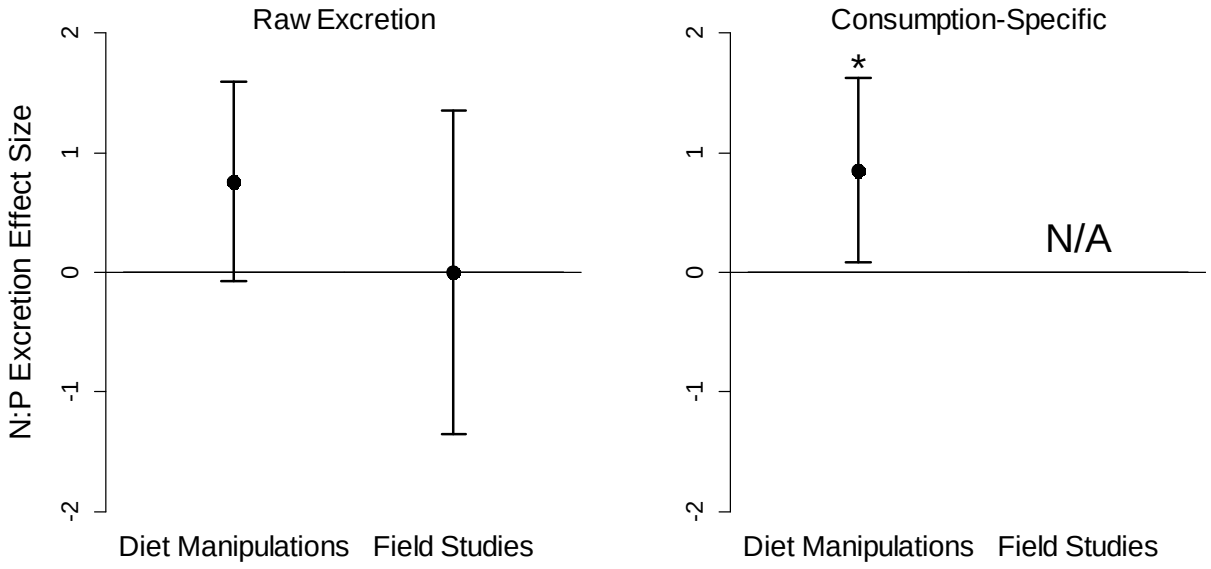


Fig. 3 Effect size of diet N:P on raw and consumption-specific N and P excretion in diet manipulation studies. Effect size, η^2, was measured as the treatment sum-of-squares divided by total sum-of-squares from a linear model then transformed into a *Z* score for ease of analysis. Consumption-specific excretion was calculated as the excretion measure presented in the study divided by mass-specific consumption rate. Points with * indicates effect size significantly different from zero based on a two-tailed *t*-test. Points indicate mean effect sizes and error bars represent 95% confidence intervals.



608
609 Fig. 4 Mean ± 95% confidence interval of effect size of diet N:P on excretion N:P. Effect size,
610 η^2, was measured as the treatment sum-of-squares divided by total sum-of-squares from a linear
611 model then transformed into a *Z* score for ease of analysis. Consumption-specific effect sizes are
612 missing in field studies because those studies did not measure consumption rate. Points with *
613 indicates effect size significantly different from zero based on a two-tailed *t*-test. Points indicate
614 mean effect sizes and error bars represent 95% confidence intervals.

615